

IN THE SPECIFICATION:

At page 1, lines 6-7, please further amend the continuing to read as follows:

-- This application is a continuation of U.S. application serial no. 08/702,502, which is the §371 U.S. national phase prosecution of PCT international application serial no. PCT/US95/02633, filed March 3, 1995, now abandoned, which is a continuation-in-part of U.S. application serial no. 207,526, filed March 7, 1994, now abandoned. --.

IN THE CLAIMS:

Please cancel claims 2, 3, 4 and 42, without prejudice.

Please amend claim 1, with the clean version provided below to read as follows:

1(Three Times Amended). A polynucleotide which, upon *in vivo* introduction into a mammalian cell, is non-replicating and induces the co-expression in the cell of at least two gene products, comprising:

a) a first transcriptional promoter which operates in eukaryotic cells upstream from, and in transcriptional control of, a first cistron, wherein the first cistron encodes at least one immunogenic epitope of a human immunodeficiency virus antigen;

b) a second cistron downstream from the first cistron, under transcriptional control either of the first transcriptional promoter or under control of a second transcriptional promoter;

c) optionally, a third cistron downstream from the second cistron, under transcriptional control either of the first transcriptional promoter or under control of the second transcriptional promoter, or under control of a third transcriptional promoter; and

d) a transcriptional terminator following each of the first, second and third cistron, unless said first cistron or second cistron is followed by a second cistron or third cistron, respectively, which lacks its own transcriptional promoter.

Please amend claim 5, with the clean version provided below to read as follows:

5(Amended). The polynucleotide of Claim 1 wherein the first cistron encodes a human immunodeficiency virus (HIV) gene selected from the group consisting of env, gag, gag/pol, gag/protease, gag and portions of pol not encoding a functional polymerase, and pol.

Please amend claim 12, with the clean version provided below to read as follows:

12(Amended). The polynucleotide of Claim 1 wherein the first cistron encodes a REV-independent human immunodeficiency (HIV) epitope, the second cistron encodes a cytokine, and the third cistron encodes a T-cell costimulatory element, wherein the first, second and third cistron may be presented in any combination.

Please amend claim 19, with the clean version provided below to read as follows:

19(Amended). The polynucleotide of Claim 18 wherein the HIV immunogenic epitope is selected from the group of HIV genes consisting of gag, gag-protease, and env or an immunogenic subportion thereof; the cytokine is interleukin-12, and the T-cell costimulatory element is a B7 protein.

Please amend claim 20, with the clean version provided below to read as follows:

20(Amended). The polynucleotide of Claim 19 wherein the env immunogenic epitope is selected from the group consisting of HIV gp160, HIV gp120 and HIV gp41.

Please amend claim 25, with the clean version provided below to read as follows:

25(Amended). A method for co-expression in a single cell *in vivo*, of at least two gene products, which comprises introducing between about 1 ng and about 100 mg of the polynucleotide of Claim 1 into the tissue of the mammal.

Please amend claim 35, with the clean version provided below to read as follows:

35(Three Times Amended). A polynucleotide which is non-replicating in eukaryotic cells *in vivo*, comprising:

- a) a eukaryotic transcriptional promoter;
- b) an open reading frame 3' to the transcriptional promoter encoding an immunogenic HIV epitope wherein the open reading frame has a splice donor sequence at the 5'-side of the open reading frame, a REV responsive element anywhere within the open

reading frame, and a stop codon encoding the termination of translation of the open reading frame;

c) an internal ribosome entry site (IRES) 3' to the translation stop codon of the open reading frame;

d) an open reading frame encoding a spliced HIV REV gene at the 3' end of which is a translation stop codon;

e) optionally, 3' to the REV translation stop codon, a second IRES, followed by an open reading frame encoding immunomodulatory or immunostimulatory genes being selected from the group consisting of GM-CSF, IL-12, interferon, and a B7 protein; and,

f) a transcription-termination signal 3' of the most downstream open reading frame of step d) or optionally, step f).

Please amend claim 44, with the clean version provided below to read as follows:

44(Twice Amended). A polynucleotide which, upon *in vivo* introduction into a mammalian cell, is non-replicating and induces the co-expression in the cell of at least two gene products, the polynucleotide comprising a first transcriptional promoter which operates in eukaryotic cells upstream from, and in transcriptional control of, a first cistron, a second cistron downstream from the first cistron, under transcriptional control either of the first transcriptional promoter or under control of a second transcriptional promoter, optionally, a third cistron downstream from the second cistron, under transcriptional control either of the first transcriptional promoter or under control of the second transcriptional promoter, or under control of a third transcriptional promoter, and a transcriptional terminator following each of the first, second and third cistron, unless said first cistron or second cistron is followed by a second cistron or third cistron, respectively, which lacks its own transcriptional promoter; wherein each of the first, second and optionally third cistrons encode a combination of any two to three of the following:

- 1) tPA-gp120_{MN};
- 2) gp160_{IIIB}/IRES/REV_{IIIB};
- 3) gp160_{IIIB};
- 4) REV_{IIIB}
- 5) tat/REV/gp160;
- 6) REV/gp160;
- 7) gp160_{MN};
- 8) gp160 from a clinical HIV isolate;
- 9) nef, using the gene from a clinical HIV isolate;

- 10) *gag*_{IIIIB};
- 11) tPA-gp120_{IIIIB};
- 12) gp160 with structural mutations selected from the group consisting of V3 loop substitutions from a clinical HIV isolate; several mutations on several constructs such variable loop removal, Asn mutations to remove steric carbohydrate obstacles to structural, neutralizing antibody epitopes; and CD4 binding site knockout mutants;
- 13) gp41 with a signal peptide leader sequence;
- 14) *gag/REV*/gp160;
- 15) *rev*: for gp160 and *gag* dicistronics;
- 16) a nucleotide sequence encoding B7;
- 17) a nucleotide sequence encoding GM-CSF;
- 18) a nucleotide sequence encoding interleukin sequences; and,
- 19) a nucleotide sequence encoding tumor associated antigens;

Please amend claim 45, with the clean version provided below to read as follows:

45(Amended). A polynucleotide construct selected from the group consisting of VIJns-(tat/rev SD), VIJns-gp160_{IIIIB}/IRES/rev_{IIIIB} (SD), VIJns-gag-prt_{IIIIB} (SD), VIJns-gag-prt_{IIIIB}, VIJns-tPA, VIJns-tPA-gp120_{MN}, VIJ-SIV_{MAC251p28} gag, VIJ-SIV_{MAC251}nef, and VIJns-tat/rev/env.

Please add new claim 46-49, as follows:

46(New). A polynucleotide which is non-replicating in eukaryotic cells *in vivo* and induces anti-HIV neutralizing antibody, HIV specific T-cell immune responses, or protective immune responses upon introduction into vertebrate tissue, including human tissue *in vivo*, wherein the polynucleotide comprises a gene encoding a gene product selected from the group consisting of HIV gag, HIV gag-protease, and HIV env, the gene containing a REV responsive element (RRE), the gene being operatively linked with a transcriptional promoter suitable for gene expression in a mammal, the gene being linked with an internal ribosome entry site (IRES), and the IRES being linked with a second gene, the second gene encoding a REV gene product.

47(New). The polynucleotide of claim 39 wherein the HIV gene open reading frame is an HIV *gag* gene or portion thereof which encodes a *gag* immunogenic epitope.